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Simultaneous Estimation of Lamivudine, Didanosine and Efavirenz in Bulk and their Formulation by UPLC

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ABSTRACT

Objectives: To develop a simple, rapid, reliable and precise RP-UPLC method for the simultaneous estimation of lamivudine, didanosine and efavirenz in bulk and its tablet dosage forms. **Materials and Methods:** The chromatographic separation was achieved by using Zorbax SB Cyano (150x4.6mm ID) 1.7µm column, isocratic mobile phase consists; Ammonium acetate Buffer pH 4.5: Acetonitrile (55:45) %v/v with a flow rate of 1.0 ml/min. The detection was carried out at 274 nm. The current method was validated according to the ICH guidelines for accuracy, precision, linearity, specificity, robustness and ruggedness. **Results:** The retention times obtained for lamivudine, didanosine and efavirenz were 2.584, 4.228 and 5.206 min respectively. The calibration curves of peak area versus concentration, were linear from 15-45µg/mL, 30-90µg/mL and 20-60µg/mL for lamivudine, didanosine and efavirenz and had regression coefficient (R^2) 0.999. Limit of detection were found to be 1.7, 3.3, 3.8 µg/ml and limit of quantification were found to be 5.1, 6.7, 11.5 µg/ml respectively for lamivudine, didanosine and efavirenz. The % assay of the marketed dosage form was found to be 100.2, 100.4 and 100.6 for lamivudine, didanosine and efavirenz. **Conclusion:** The experimental study results revealed the suitability of proposed method that can be used for simultaneous estimation of lamivudine, didanosine and efavirenz in bulk and their pharmaceutical formulations for routine quality control analysis.

Keywords: Lamivudine, Didanosine, Efavirenz, Ultra performance liquid chromatography, Validation.

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INTRODUCTION

High-speed chromatographic separations such as ultra-performance liquid chromatography (UPLC) have been considered as one of the important developments in the area of fast chromatographic separations with reduced analysis time and good efficiency.[1]

Ultra-Performance Liquid Chromatography (UPLC) is relatively a modern technique which gives a new direction for liquid chromatography and utilizes particles having less than 2 µm in diameter operating at elevated mobile phase velocities to result in better resolution, speed and sensitivity as compared with High-Performance Liquid Chromatography (HPLC). It uses fine particles and saves time and reduces solvent consumption. The UPLC system reduces analysis time up to nine times compared to the conventional system using 5µm particle packed analytical columns. In UPLC the separation is performed under tremendous pressures (upto 100 MPa is possible), but it has no negative impact on analytical column as well as other components of chromatographic system. Separation efficiency remains maintained and also it is even improved more.[2-6]

Antiviral drugs are most commonly designed with the purpose of combating various human infecting viruses such as HIV, herpes, hepatitis and influenza. To counter severe conditions, antiviral drugs are administered in combination, usually three to four; this regimen is known as highly active antiretroviral therapy.[7-9] Lamivudine (3TC) is a nucleoside analogue reverse transcriptase inhibitor that has been widely used against HIV infection which also has antiviral effects against hepatitis B.[10,11] Lamivudine is the *levo* (–) enantiomer of a cytidine analogue with sulfur substituted for the 3′ carbon atom in the furanose ring [(–) 2′,3′-dideoxy, 3′-thiacytidine].[12] It is a white to beige powder with a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.26 g/mol. Its chemical structure is given in Figure 1. Didanosine (2′, 3′-dideoxyinosine) is a purine nucleoside with inhibitory activity against both HIV-1 and HIV-2. It is a fluffy white solid or powder with a molecular formula of $C_{10}H_{12}N_4O_3$ and a molecular weight of 236.23 g/mol. Its chemical structure is given in Figure 2. Efavirenz is a non-nucleoside reverse transcriptase inhibitor with excellent inhibitory activity against HIV-1.[13] Efavirenz is chemically described as (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one.[14] It is a white to slightly pink crystalline powder with a molecular formula of $C_{14}H_9ClF_3NO_2$ and a molecular weight of 315.67 g/mol. Its chemical structure is given in Figure 3.

The literature survey reveals that a few spectroscopic and liquid chromatographic procedures have been reported for the determination of lamivudine, didanosine and efavirenz by ultraviolet (UV) and high-performance liquid chromatography (HPLC) and no UPLC method was reported [Raja *et al*., Naga Sandhya *et al*.,].[15,16] Therefore, we proposed to develop a rapid, sensitive UPLC method for simultaneous estimation of lamivudine, didanosine and efavirenz in bulk and pharmaceutical dosage form.

Present study involves development and validation of UPLC method for the simultaneous estimation of lamivudine, didanosine and efavirenz in bulk and combined tablet dosage form, which is fast, sensitive with better resolution and peak symmetry. Finally, the developed method was validated as per ICH guidelines.

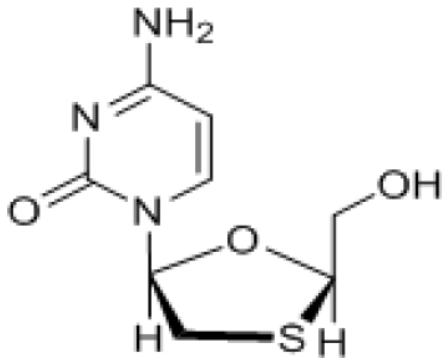

Figure 1: Chemical structure of Lamivudine.

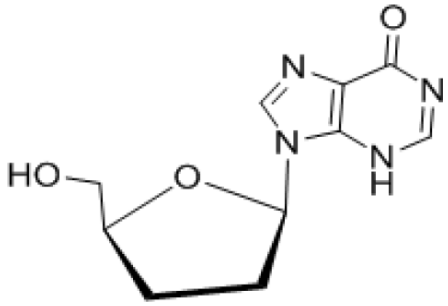

Figure 2: Chemical structure of Didanosine.

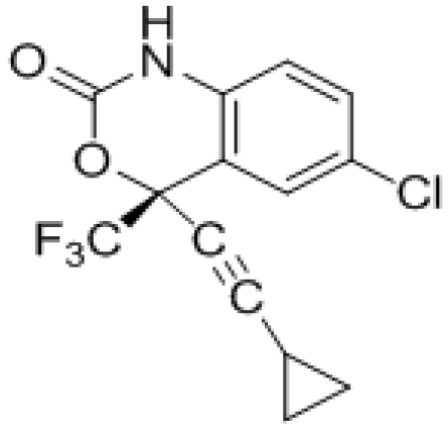

Figure 3: Chemical structure of Efavirenz.

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards of lamivudine, didanosine and efavirenz were provided as a gift sample from Chandra labs, Hyderabad. ODIVIR tablets labelled to contain lamivudine 300 mg, didanosine 400 mg, and efavirenz 600 mg were purchased from the local pharmacy store. HPLC grade acetonitrile was purchased from Merck, Hyderabad. Ammonium acetate Buffer AR Grade was purchased from Rankem, Mumbai, India. HPLC grade water was used throughout the process, which was prepared using Millipore MilliQ water purification system.

UPLC Method Development

Instrumentation

The instruments used in the study were electronic balance (Sigma200), sonicator (PCi, 3.5 L) and digital pH meter (Unilab). Agilent UPLC system model 1290 equipped with 1700 model UV detector, auto sample injector, and column Zorbax SB Cyano (150x4.6mm ID) 1.7µm, respectively. The output signal was monitored and integrated using chemistation software.

Chromatographic conditions

The present assay was carried out on Agilent UPLC system model 1290 equipped with 1700 model uv detector, auto sample injector, and column Zorbax SB Cyano (150x4.6mm ID) 1.7µm, respectively. The output signal was monitored and integrated using chemistation software. The isocratic mobile phase consisted of Ammonium acetate Buffer pH 4.5: Acetonitrile (55:45) % v/v, flowing through the Zorbax SB Cyano (150x4.6mm ID) 1.7µm column at a constant flow rate of 1.0 ml/ min at ambient temperature. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min with a sample injection volume of 20µl. Detection of the analytes was carried out at a wavelength of 274 nm.

Determination of Working Wavelength (λ_{max})

In simultaneous estimation of triple drugs, isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation, to estimate the drugs accurately.

Preparation of Mobile phase

20 Mm Buffer preparation: Accurately weighed and transferred 1.54 g of Ammonium acetate into a 1000 mL volumetric flask, to this 500mL of Milli-Q water was added and sonicated to dissolve and made up the volume with Milli-Q water and pH was adjusted to 4.5 with 1mL of triethanolamine.

Mobile phase: About 450mL of HPLC grade acetonitrile was added to 550mL of buffer solution and sonicated for 10 mins (55:45% v/v).

Preparation of Standard stock solution for UV

About 10 mg each of Lamivudine (3TC), didanosine (ddI) and efavirenz (EFV) were weighed and transferred into three different 50 mL volumetric flask, to each 20 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase so the concentration of 3TC, ddI and EFV would be 200 µg/mL each**.**

Dilutions

Necessary dilutions (2.5ml in 50ml) are made from standard stock solutions to get the concentration range of 10 µg/mL of 3TC, 10 µg/mL of ddI and 10µg/mL of EFV.

The wavelength of maximum absorption (λ_{max}) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 202 & 272 nm for 3TC (Figure 4), 223 & 274 nm for ddI (Figure 5) and 239 & 319 nm for EFV (Figure 6). Thus 274 nm was selected as detector wavelength at which three drugs are having absorbance for the UPLC chromatographic method.

Selection and preparation of mobile phase

Various mobile phases containing methanol, water, acetonitrile, phosphate buffer, ammonium acetate buffer and triethylamine in different ratios were tried with different flow rates. Good symmetrical peak was found with the mobile phase comprising ammonium acetate buffer and acetonitrile in the ratio 55:45 (v/v) (pH adjusted to 4.5 with triethylamine). Mobile phase was prepared by mixing about 450mL of

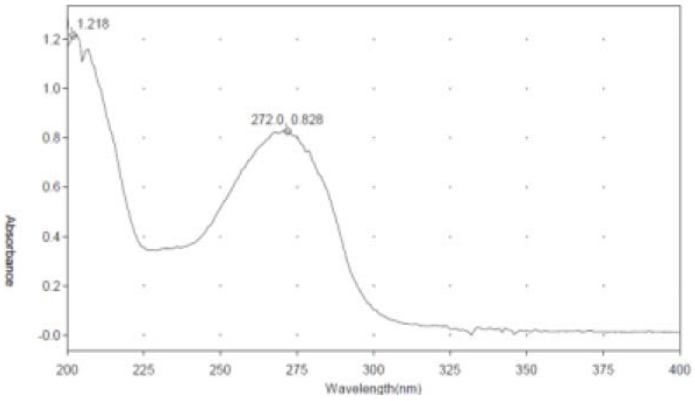

Figure 4: UV absorbance spectra for lamivudine.

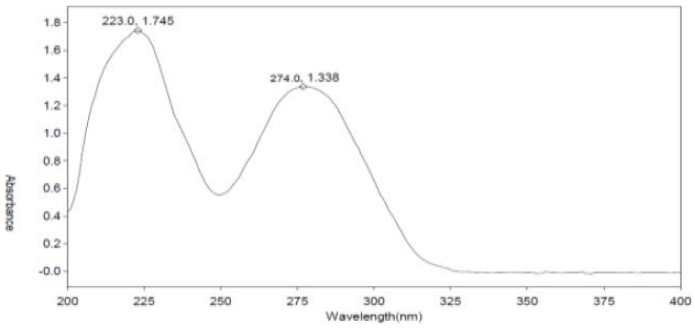

Figure 5: UV absorbance spectra for didanosine.

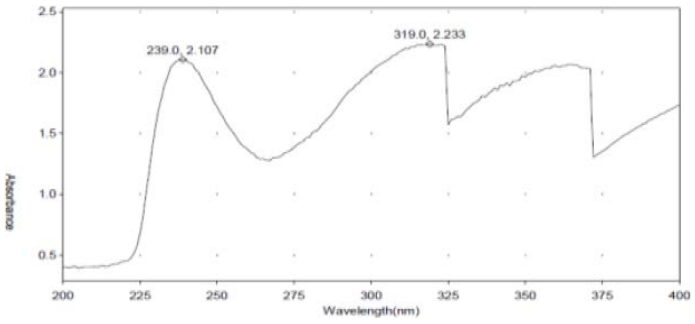

Figure 6: UV absorbance spectra for efavirenz.

HPLC grade acetonitrile and 550mL of buffer solution and sonicated for 10 mins (55:45% v/v), and the pH was adjusted to 4.5 with triethylamine. The mobile phase was sonicated for 10 min and filtered through the 0.45µm membrane filter.

Preparation of standard stock solutions

300 mg of lamivudine, 400 mg of didanosine, and 600 mg of efavirenz working standards were weighed and transferred into a 100ml clean dry volumetric flask and 70 ml of mobile phase was added to this flask and sonicated for 30 min and made up the final volume with mobile phase and labelled as standard stock solution-I containing 3mg/ml of LMV, 4mg/ml of ddI and 6mg/ml of EFV. 5 mL of the standard stock-I pipetted in to 50 mL volumetric flask and made-up volume with mobile phase (Standard solution II). 5 mL of the standard stock-II pipetted into 50 mL volumetric flask and made-up volume with mobile phase (Working Standard solution). The final concentration of Working Standard solution would be 30 µg/ml lamivudine, 40 µg/ml didanosine, and 60 µg/ml efavirenz. The resulting solution is used to record the chromatogram (Figure 7).

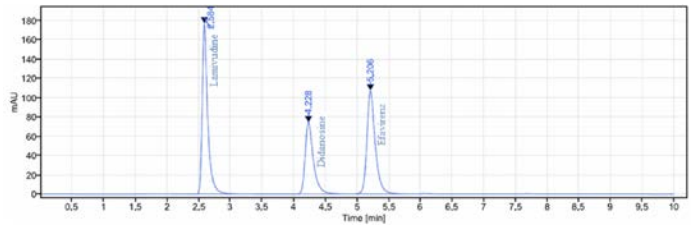

Figure 7: Typical optimized chromatogram of lamivudine, didanosine and efavirenz.

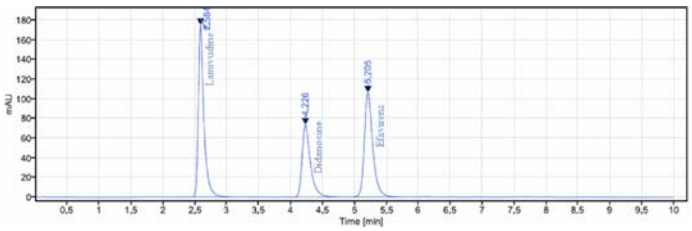

Figure 8: Typical formulation chromatogram of lamivudine, didanosine and efavirenz.

Preparation of Sample Solution

Twenty tablets of 3TC, ddI and EFV were weighed and calculated average weight, then tablets were crushed into fine powder with mortar and pestle. The powder equivalent to 300 mg of 3TC, 400mg of ddI and 600mg of EFV were transferred into 100 mL volumetric flask and added 70mL of mobile phase then sonicated it for 30min with intermittent shaking, after 30min made up the volume with mobile phase (Sample Stock-I), then centrifuged the sample at 5000RPM for 10min.Pipetted 5 mL upper clear sample stock-I solution into 50 mL volumetric flask and made-up volume with mobile phase (Sample Stock-II).

Pipetted 5 mL of the sample stock-II into 50 mL volumetric flask and made-up volume with mobile phase (working sample solution). Filter the solution through 0.45µm filter paper. The final concentration of Working Sample solution would be 30 µg/ml lamivudine, 40 µg/ml didanosine, and 60 µg/ml efavirenz. The resulting solution is used to record the chromatogram (Figure 8).

Preparation of calibration curve

2.5, 3.75, 5.0, 1.0, 6.25, and 7.5 ml of standard solution-II was transferred into a 50 mL volumetric flask and diluted up to mark with mobile phase. So, the final concentrations were in the range of 15-45 µg/ml lamivudine, 20-60 µg/ml didanosine, and 30-90 µg/ml efavirenz. The calibration standard solution of lamivudine, didanosine and efavirenz were injected using a 20µl injector and the chromatograms were recorded at 274 nm. Evaluation of the drugs was performed and peak areas were recorded. Calibration curves were constructed by plotting the peak area on the y-axis against respective concentration of the drug on the x-axis. The calibration curve was evaluated by its coefficient of determination (R^2).

Optimized Chromatographic Conditions

After systematic and detailed study of the various parameters involved in the method, the following were found to be optimized conditions and employed for further studies: Table 1

Methods Validation

The developed method for lamivudine, didanosine and efavirenz was validated for parameters such as system suitability, linearity and range, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), filter compatibility and solution stability as per ICH guidelines.

Table 1: Optimized chromatographic conditions.

Instrument	Agilent UPLC system model 1290
Mobile phase	20 mM Ammonium acetate Buffer pH 4.5: Acetonitrile (55:45) %v/v
Column	Zorbax SB Cyano (150x4.6mm ID) 1.7µm
Flow rate	1.0 mL/min
Column temperature	25°C
Sample temperature	25°C
Wavelength	274 nm
Injection volume	20µL
Run time	10min
Retention time	2.584 min, 4.228 min and 5.206 min

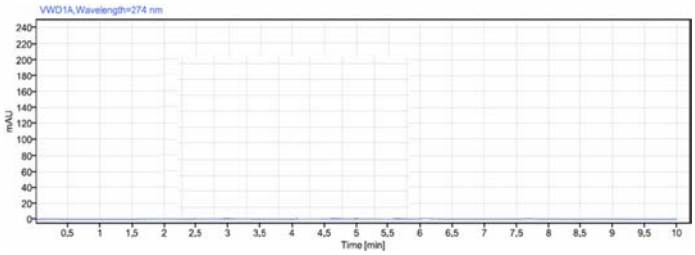

Figure 9: A typical chromatogram of lamivudine, didanosine and efavirenz blank.

System Suitability

To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like asymmetry factor, theoretical plate number (N), resolution (Rs) and retention time (t_R) were analyzed by using 20µL of the working standard solution containing 30µg/mL of 3TC, 40µg/mL of didanosine and 60µg/mL of EFV were injected six times into UPLC system and the chromatograms were recorded for the same.

Specificity

It is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might be including impurities, degradants, or matrix.

Blank solution was injected and the chromatogram was recorded (Figure 9). Placebo solution was prepared and it was injected and the chromatogram was recorded (Figure 10).

Linearity and Range

For establishing linearity, 2.5, 3.75, 5.0, 1.0, 6.25, and 7.5 ml of working standard solution containing 300 µg/ml of lamivudine, 400 µg/ml of didanosine, and 600 µg/ml of efavirenz was pipetted out and transferred into a 50 mL volumetric flask of concentration and these are diluted up to mark with mobile phase. So, the final concentrations were in the range of 15-45 µg/ml lamivudine, 20-60 µg/ml didanosine, and 30-90 µg/ml efavirenz. The calibration standard solution of lamivudine, didanosine and efavirenz were injected using a 20µl injector and the chromatograms were recorded at 274 nm and calibration curve was constructed by plotting the peak area versus drug concentration.

LOD and LOQ

A study to establish the LOD and LOQ for lamivudine, didanosine and efavirenz was conducted. Series of very dilute LOD and LOQ solutions were prepared as per the test method and injected triplicate into the UPLC system. The LOD and LOQ were established based on signal-to-noise ratio. LOD was established by identifying the concentration which showed s/n ratio of 3, whereas LOQ was established by identifying the concentration which gave s/n ratio of about 10.

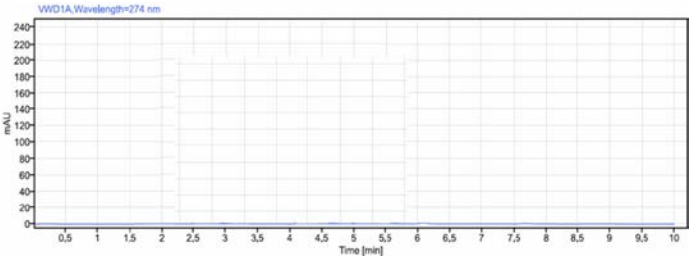

Figure 10: A typical chromatogram of Placebo.

Accuracy

Accuracy of the method was determined by recovery studies. A known amount of lamivudine, didanosine and efavirenz at each three concentration levels 50%, 100%, and 150% was added to a pre-analyzed sample solution (formulation) and injected in triplicate at each level into the UPLC system. The percentage recovery and mean percentage recovery of lamivudine, didanosine and efavirenz at each level was calculated.

Method precision

Method precision was determined by injecting six different working sample solutions of 3TC (30µg/mL), EFV (60µg/mL) and didanosine (40µg/mL) into UPLC system and chromatograms were obtained. The %RSD of the assay result of six preparations was calculated.

Intermediate Precision (Ruggedness) / interday precision

Intermediate precision (also called within-laboratory or within-device in different days, different analysts) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similar objects over an extended period of time. The Intermediate Precision (Ruggedness) was determined by injecting six different working sample solutions of 3TC (30µg/mL), EFV (60µg/mL) and didanosine (40µg/mL) into UPLC system and chromatograms were obtained. The %RSD of the assay result of six preparations of different analysts was calculated. Table 10.

Robustness

Working standard solution was injected into the UPLC system at variable conditions such as column temperature ± 5°C and wavelength by ± 5nm.

ASSAY

The commercial tablet (ODIVIR) was analyzed by injecting 6 replicates of standard and sample working solutions injected into the UPLC system and chromatograms were recorded. The amount of the drug present in marketed tablets was calculated by comparing the peak area of standard and sample. The % assay of lamivudine, didanosine and efavirenz were found to be 98–102%. The results of assay are shown in Table 14.

$$\% \text{ Assay} = \frac{AT \times WS \times 5 \times 5 \times DT \times 50 \times 50 \times P \times AW \times 100}{AS \times DS \times 50 \times 50 \times WT \times 5 \times 5 \times 100 \times LC}$$

Where,

AS: Average peak area due to standard preparation

AT: Average peak area due to sample preparation

WS: Standard Weight of 3TC/didanosine/EFV in mg

WT: Weight of sample in preparation

DT: Dilution of assay preparation

DS: Dilution of standard preparation

P: Purity of 3TC/didanosine /EFV

AV: Average weight of tablets in mg

LC: Labelled claim of 3TC/didanosine/EFV

Filter compatibility

Filter compatibility was determined by injecting unfiltered and filtered working sample solution through 0.45µm PVDF (polyvinylidene fluoride) and 0.45µm Nylon individually into UPLC system by discarding the 2mL of filtrate. The difference between unfiltered sample % Assay and filtered sample % Assay should not be more than 2.0%. Filter compatibility results are shown in Table 15.

Solution stability of the standard and sample

Solution stability of the standard and sample was determined by injecting working standard solution and working sample solution into UPLC system at 12hr and 24hr of time intervals. Results of solution stability are shown in Table 16-17.

RESULTS

System Suitability

The column efficiency for 3TC, didanosine and EFV peaks was identified from the theoretical plate count of more than 3000 and the tailing factor was less than 2.0. %RSD for peak areas from six replicate injections was found to be less than 2.0 %. The results of other system suitability parameters, such as, resolution, peak tailing, and theoretical plates, are presented in Table 2. All system-suitable parameters were found to be satisfactory.

Linearity

Linearity was evaluated by analyzing different concentrations. The correlation coefficient obtained was greater than 0.999 for all the components. The slope and y-intercept values were also provided in Table 3, which confirmed good linearity between peak areas and concentration. The linearity graphs of 3TC, didanosine and EFV are shown in Figs 11, 12 and 13 respectively.

LoD and LoQ

The Limit of Detection and Limit of Quantification of 3TC, didanosine and EFV were calculated by using the following equations (ICH, Q2 (R1)). The LoD and LoQ values are reported in Table 4, 5.

These LoD = 3.3 × σ/S and LoQ = 10 × σ/S

Where σ = the standard deviation of the response and S = slope of the calibration curve.

Accuracy

The %recovery for 3TC, didanosine and EFV was within the range of 98–102%. The %RSD for 3TC, didanosine and EFV was found to be

Table 2: System suitability test parameter.

Sl.No.	Peak name	Retention time (Minutes)	Peak area	Theoretical plates	Tailing factor	Resolution
1.	Lamivudine	2.58	1078.31	39751	1.6	
2.	Didanosine	4.23	672.49	45781	1.5	5.2
3.	Efavirenz	5.20	979.27	64456	1.3	4.2

Table 3: Linearity data results.

Lamivudine		Didanosine		Efavirenz	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
15	752.49	20	217.06	30	359.07
22.5	922.15	30	430.25	45	684.04
30	1100.25	40	674.9	60	986.53
37.5	1283.1	50	900.9	75	1293.53
45	1450	60	1156.12	90	1605.36
Regression equation		Regression equation		Regression equation	
y=23.41x+399.2		y=23.48x-263.6		y=20.68x-255.1	
Square of correlation coefficient (R^2) = 0.9992		Square of correlation coefficient (R^2) = 0.9991		Square of correlation coefficient(R^2=0.9997)	

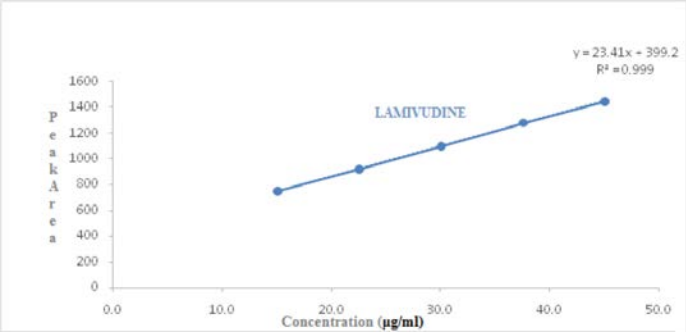

Figure 11: Standard calibration curve of lamivudine.

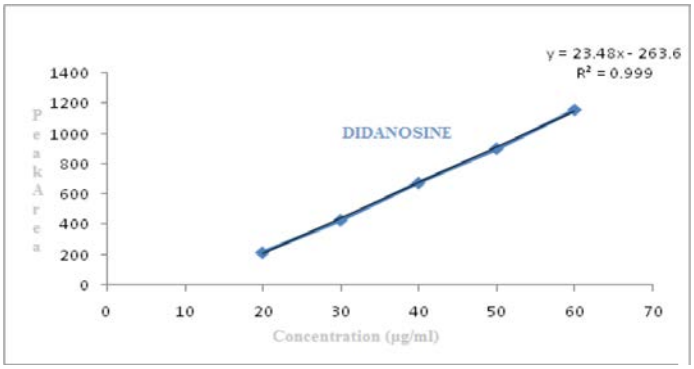

Figure 12: Standard calibration curve of didanosine.

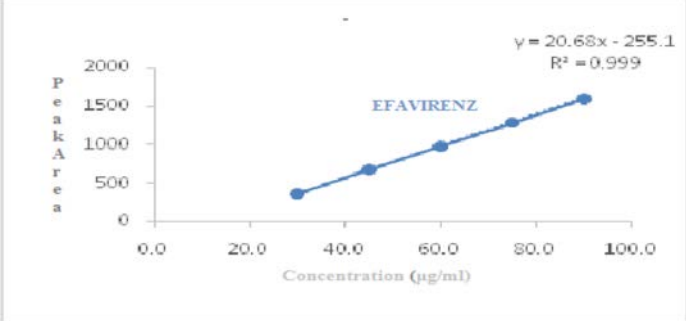

Figure 13: Standard calibration curve of efavirenz.

Table 4: Limit of detection and Limit of quantification results.

Sl. No.	Parameter	Measured values (µg/ml)		
		Lamivudine	Didanosine	Efavirenz
1.	Limit of detection	1.7	3.3	3.8
2.	Limit of quantification	5.1	6.7	11.5

Table 5: Precision at LOQ.

Injection	Lamivudine	Didanosine	Efavirenz
	Area	Area	Area
1.	185.70	113.22	187.32
2.	183.55	113.51	186.42
3.	185.62	112.59	185.19
4.	185.63	116.05	186.71
5.	187.42	114.61	186.49
6.	184.69	115.32	186.27
AVG	185.44	114.22	186.40
% RSD	0.7	1.2	0.4

Table 6: % recovery results of lamivudine.

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	15	15.3	101.9	100.8
	15	15.3	101.7	
	15	15.3	101.9	
100%	30	30.1	100.2	
	30	30.2	100.6	
	30	30.1	100.2	
150%	45	45.1	100.2	
	45	45.2	100.4	
	45	45.2	100.5	

Table 7: % recovery results of didanosine.

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	20	19.6	98.1	99.3
	20	20.0	100.2	
	20	20.2	100.9	
100%	40	40.1	100.2	
	40	40.2	100.5	
	40	40.0	100.1	
150%	60	61.2	102.0	
	60	61.1	101.9	
	60	60.8	101.3	

Table 8: Mean % recovery results of efavirenz.

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	30	29.1	97.1	99.3
	30	30.1	100.2	
	30	29.9	99.8	
100%	60	60.1	100.2	
	60	60.2	100.3	
	60	60.1	100.2	
150%	90	88.6	98.5	
	90	88.8	98.7	
	90	89.0	98.9	

Table 9: Method precision results

Injection. No.	% Assay of drugs		
	Lamivudine	Didanosine	Efavirenz
1.	101.11	100.59	100.57
2.	100.47	100.34	100.25
3.	100.70	100.45	100.26
4.	100.19	100.46	100.10
5.	100.83	100.52	100.14
6.	100.71	100.26	100.10
Average	100.7	100.2	100.4
% RSD	0.2	0.1	0.3

Table 10: Intermediate precision results.

	% Assay of drugs		
	Lamivudine	Didanosine	Efavirenz
Analyst-1	100.7	100.4	100.2
Analyst-2	101.0	99.1	100.9
Average	100.9	99.8	100.6
% RSD	0.2	0.9	0.5

within 2%. Hence the proposed method was accurate, and the results are summarized in Table 6, 7 and 8.

Method Precision

%Assay for 3TC, didanosine and EFV were in the range of 98–102%. The %RSD for 3TC, didanosine and EFV were found to be within 2%. Hence the method is precise, reproducible, and rugged for 48 hr study and the results are summarized in Table 9.

Robustness

The system suitability parameters such as resolution, tailing factor and theoretical plates of 3TC, didanosine and EFV remained unaffected

Table 11: Robustness results of Lamivudine.

Chromatographic	changes	Theoretical Plates	Tailing factor	Resolution
Temperature ± 5°C	20	39854	1.6	-
	30	39584	1.6	-
Wavelength ± 5nm	269	39851	1.5	-
	279	39885	1.5	-

Table 12: Robustness results of Didanosine.

Chromatographic	changes	Theoretical Plates	Tailing factor	Resolution
Temperature (± 5°C)	20	45584	1.5	5.6
	30	45725	1.5	5.6
Wavelength (± 5nm)	269	45733	1.5	5.6
	279	45725	1.5	5.6

Table 13: Robustness results of Efavirenz.

chromatographic	changes	Theoretical Plates	Tailing factor	Resolution
Column Temperature (± 5°C)	20	30040	1.3	4.2
	30	30014	1.3	4.2
Wavelength (nm) (± 5nm)	269	30533	1.3	4.2
	279	30458	1.3	4.2

Table 14: Assay results.

Drug	Lamivudine	Didanosine	Efavirenz
Label claim (mg)	300	400	600
Amount found (mg)	300.6	401.2	603.6
% Assay	100.2	100.4	100.6

Table 15: Filter compatibility results.

Analyte	Initial	PVDF 0.45µm	%Difference	NYLON 0.45µm	%Difference
	%Assay			%Assay	%Assay
Lamivudine	100.2	101.1	0.9	99.4	0.8
Didanosine	100.4	100.3	0.1	100.1	0.3
Efavirenz	99.6	99.8	0.2	100.3	0.7

Table 16: Results of solution stability of standard.

Analyte	Initial Area	12Hrs Area	% Difference	24Hrs Area	% Difference
Lamivudine	1079.4	1077.11	0.2	1066.85	1.2
Didanosine	672.68	670.41	0.3	670.52	0.3
Efavirenz	979.24	980.15	0.1	979.11	0.0

Table 17: Results of solution stability of sample.

Analyte	Initial /Area	12Hrs/ Area	%Difference	24Hrs/ Area	%Difference
Lamivudine	1090.3	1089.52	0.1	1084.52	0.5
Didanosine	676.45	675.44	0.1	675.62	0.1
Efavirenz	984.82	981.36	0.4	987.62	0.3

by deliberate changes. The results were presented in Table 11, 12 and 13. Thus, the method was found to be robust concerning variability in applied conditions

DISCUSSION

In this UP–HPLC method, the linearity was within the range of 10–90 µg/mL, the method was successfully validated in the optimized conditions, and the results of validation for various parameters were within the limits. The linearity of this method was established in the concentration range of 15 to 45µg/mL, 20 to 60µg/mL and 30 to 90µg/mL for lamivudine, didanosine and efavirenz. Dilution and filtration were only required for sample preparation. The quantification was achieved by using a Zorbax SB Cyano (150x4.6mm ID) 1.7µm column and an isocratic mobile phase consisting of Ammonium acetate Buffer pH4.5: Acetonitrile (55:45) %v/v. The flow rate was 1.0 ml/min. Retention times were 2.584 min, 4.226 min and 5.205 min for lamivudine, didanosine and efavirenz. The lower limit of quantification (LOQ) and lower limit of detection (LOD) for lamivudine, didanosine and efavirenz were found to be 5.1µg/ml, 6.7µg/ml, 11.5µg/ml and 1.7µg/ml, 2.2µg/ml, 3.8µg/ml respectively. The mean percentage recovery of lamivudine, didanosine and efavirenz were 100.3, 99.3 and 99.3. Filter compatibility was determined and difference between unfiltered sample % Assay and filtered sample % Assay was not more than 2.0%. The method was validated according to ICH guidelines. The developed method has been successfully applied for the determination of lamivudine, didanosine and efavirenz for APIs and tablet formulations.

CONCLUSION

A convenient, rapid, accurate and precise UPLC method was developed for the simultaneous determination of lamivudine, didanosine and efavirenz in pharmaceutical formulations. The assay provides a linear response across a wide range of concentrations. This method can be said to be more economical as compared to other methods reported in literature. The method is suitable for the determination of these drugs in tablets, and hence can be used for routine quality control of lamivudine, didanosine and efavirenz in this dosage form.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS

RP-UPLC: Reverse Phase Ultra Performance Chromatography; **ICH:** International Council for Harmonisation; **3TC:** Lamivudine; **ddI:** Didanosine; **EFV:** Efavirenz; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard Deviation.

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